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Intraspecific Competition and Inbreeding Depression: Increased Competitive Effort by Inbred Males Is Costly to Outbred Opponents

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ABSTRACT: A recent theoretical model suggests that intraspecific competition is an important determinant of the severity of inbreeding depression. The reason for this is that intraspecific competition is density dependent, leading to a stronger negative effect on inbred individuals if they are weaker competitors than outbred ones. In support of this prediction, previous empirical work shows that inbred individuals are weaker competitors than outbred ones and that intraspecific competition often exacerbates inbreeding depression. Here, we report an experiment on the burying beetle *Nicrophorus vespilloides*, in which we recorded the outcome of competition over a small vertebrate carcass between an inbred or outbred male resident caring for a brood and a size-matched inbred or outbred male intruder. We found that inbred males were more successful as intruders in taking over a carcass from a male resident and were injured more frequently as either residents or intruders. Furthermore, inbred males gained less mass during the breeding attempt and had a shorter adult life span than outbred males. Finally, successful resident males reared a substantially smaller brood comprised of lighter larvae when the intruder was inbred than when it was outbred. Our results shows that inbred males increased their competitive effort, thus contradicting previous work suggesting that inbred males are weaker competitors. Furthermore, our results shows that inbred intruders impose a greater cost to resident males, suggesting that outbred individuals can suffer fitness costs as a result of competition with inbred ones.

Keywords: competitive effort, injuries, life span, *Nicrophorus vespilloides*, reproductive success, terminal investment.

Introduction

Inbreeding depression refers to the reduction in fitness suffered by any offspring produced as a result of mating between relatives (Charlesworth and Charlesworth 1987). There is good evidence for inbreeding depression from a wide

range of animals and plants studied either in the wild or under laboratory conditions (Lynch and Walsh 1998; Crnokrak and Roff 1999). For instance, inbred individuals may suffer a decline in juvenile survival (Kruuk et al. 2002), growth (Gjerde et al. 1983), mating success (Miller et al. 1993), fecundity (Radwan 2003), and/or adult life span (Lynch and Walsh 1998). There is a growing awareness that variation in environmental conditions often influences the severity of inbreeding depression (Armbruster and Reed 2005). For instance, harsh conditions (such as extreme temperatures, toxic chemicals, and intense intraspecific competition) can exacerbate inbreeding depression (Armbruster and Reed 2005; Fox and Reed 2011), while benign conditions (such as sociality and parental care) can buffer against it (Avilés and Bukowski 2006; Pilakouta et al. 2015).

Intraspecific competition is a key component of the social environment of most animals, the outcome of which determines an individual's access to resources and/or sexual partners and thereby its growth, survival, and/or reproductive success (Huntingford and Turner 1987; Keddy 2001). Intraspecific competition may be an important determinant of the severity of inbreeding depression because it is associated with density dependence, leading to a disproportionately strong negative effect on the fitness of inbred individuals if they are the weaker competitors (Yun and Agrawal 2014). In support of this, empirical studies show that inbred individuals are weaker competitors than outbred ones (Sharp 1984; Meagher et al. 2000; Haag et al. 2002; Höglund et al. 2002; Hoffman et al. 2004; Välimäki et al. 2007) and that inbreeding depression is often more severe when there is more intense intraspecific competition (Meagher et al. 2000; Haag et al. 2002; Yun and Agrawal 2014).

Here, we identify two outstanding issues in our understanding of how intraspecific competition influences inbreeding depression. First, although previous work shows that inbred individuals are weaker competitors (Sharp 1984;

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Meagher et al. 2000; Haag et al. 2002; Höglund et al. 2002; Hoffman et al. 2004; Välimäki et al. 2007), inbred individuals might increase their competitive effort due to terminal investment if they have a lower future reproductive potential than outbred ones (Lynch and Walsh 1998). The weaker competitiveness of inbred individuals reported in prior studies might reflect that inbred individuals are often smaller on average than outbred ones (Gjerde et al. 1983) and that body size is an important determinant of the outcome of competition (Huntingford and Turner 1987). Thus, the effect of inbreeding on body size may mask any effect of inbreeding on competitive effort. Few studies account for the effect of body size, but a study on common shrews (*Sorex araneus*) found that the weaker competitive ability of inbred individuals was independent of body size (Välimäki et al. 2007). Second, previous studies have ignored potential fitness costs to outbred individuals due to competition with inbred ones. Such effects would emerge from indirect genetic effects, which occur whenever genes expressed in one individual influence the expression of a trait in another (Moore et al. 1997). Currently, little attention has been given to indirect genetic effects in the context of inbreeding (Mattey et al. 2013; Mattey and Smiseth 2015a). However, if inbred individuals increase their competitive effort, outbred opponents might suffer a fitness cost because of competition with inbred individuals. To address these issues, we need studies that control for effects of size asymmetries between inbred and outbred competitors and that record fitness consequences to opponents of inbred and outbred individuals.

We used the burying beetle *Nicrophorus vespilloides* as a model to study the interaction between intraspecific competition and inbreeding depression. This species breeds on carcasses of small vertebrates (Scott 1998). Both parents provide elaborate parental care, which includes application of antimicrobial secretions to the carcass, provisioning larvae with predigested carrion, and protecting the carcass and brood from predators and conspecific intruders (Eggert et al. 1998; Smiseth et al. 2005; Rozen et al. 2008; Arce et al. 2012). Intruders pose a substantial threat because they will kill the resident's larvae and rear their own brood if they succeed in taking over a carcass (Scott 1990; Trumbo 1991). Successful takeovers by intruders occur regularly in the wild (Scott 1990; Trumbo 1991). There is evidence for inbreeding depression as inbred individuals have higher early mortality and shorter adult life spans than outbred ones (Mattey et al. 2013; Pilakouta et al. 2015). However, there is no evidence for inbreeding depression in growth (Mattey et al. 2013; Pilakouta et al. 2015), and inbred and outbred individuals do not differ in body size. Previous work shows that inbred males provide more indirect parental care than outbred males, suggesting that inbred males increase their effort toward current reproduction (Mattey and Smiseth 2015a).

The aim of this study was to test for effects of inbreeding on intraspecific competition in *N. vespilloides*. We used a 2×3 factorial design, where an inbred or outbred male resident faced either a size-matched inbred or outbred male intruder or no intruder. If inbred males increase their competitive effort for a current reproductive opportunity, we predicted that they would be more successful in defending a carcass as residents, more successful in taking over a carcass as intruders, and more likely to be injured as residents and intruders than outbred males. Furthermore, if inbred males increase their effort toward current reproduction, we predicted that they would gain less mass while breeding, produce larger broods and/or heavier larvae, and have a shorter adult life span. Finally, if opponents suffered a fitness cost from competing with inbred males, we predicted that resident males would produce smaller broods and/or lighter larvae when the intruder was inbred than when he was outbred.

Material and Methods

General Methods

All beetles used in our experiments were from our outbred laboratory population maintained at the University of Edinburgh. The stock population descended from wild-caught beetles originally collected in Warmond (Netherlands) and Edinburgh (UK). We kept the stock population outbred by maintaining a large population each generation, outcrossing our stock population with wild-caught beetles each summer, and always mating unrelated or distantly related males and females (i.e., no shared common ancestors for two or more generations; Mattey et al. 2013; Mattey and Smiseth 2015b). All beetles were kept under constant light at 20°C. Nonbreeding adults were housed individually in transparent plastic containers (12 cm \times 8 cm \times 2 cm) filled with moist soil and fed organic beef twice a week.

To generate outbred and inbred males for our experiments, we first paired up males and females from the stock population during the previous generation. These beetles were placed in a transparent plastic container (17 cm \times 12 cm \times 6 cm) filled with 1 cm of moist soil and provided with a freshly thawed mouse carcass (supplied by Livefoods Direct, Sheffield, UK) with a mass of 20–22 g. We generated outbred individuals by mating unrelated or distantly related males and females using the protocol described above for the stock population, while we generated inbred individuals by mating females with their full brother.

Experimental Design

To investigate the interaction between intraspecific competition and inbreeding depression, we used a 2×3 factorial design with the following treatments: (1) an inbred resident

challenged by an inbred intruder ($n = 20$), (2) an inbred resident challenged by an outbred intruder ($n = 19$), (3) an inbred resident not challenged by an intruder ($n = 20$), (4) an outbred resident challenged by an inbred intruder ($n = 18$), (5) an outbred resident challenged by an outbred intruder ($n = 20$), and (6) an outbred resident not challenged by an intruder ($n = 20$). Inbred and outbred males that were not challenged by an intruder are referred to as control males because they were not exposed to competition from an intruder.

Before breeding, we weighed male beetles to obtain their pretrial mass and used digital calipers to measure the width of their pronotum as a measure of body size (Müller et al. 1990). We matched residents and intruders for body size by ensuring that they had a pronotum width within $\pm 10\%$ (mean difference in pronotum width \pm SE = $2.96\% \pm 0.0036\%$). We did this because body size is a strong determinant of competitive ability in *Nicrophorus* beetles (Otronen 1988; Safryn and Scott 2000). We also ensured that males were of a similar age to control for any age-related differences in competitive effort. Given that residents and intruders were matched for size, we marked them by applying either one or two small spots of correction fluid on the elytra. This method of marking is short lasting and nontoxic and has no discernible effect on behavior (Hagler and Jackson 2001). To exclude any potential effect of marking on the outcome of the trials, we alternated which of the two males (i.e., resident or intruder) was given two spots between experimental blocks. All beetles were virgins and were uninjured at the start of the experiment.

We paired all inbred or outbred resident males to unrelated outbred females and allowed them to breed, following the same protocol used to generate experimental beetles. We checked the pairs regularly, and when first-instar larvae were present on the carcass (usually 3–4 days after pairing), we removed the female. We then introduced either an inbred or an outbred male intruder in experimental treatments or no intruder in the two control treatments. Females were removed because females contribute toward brood defense (Trumbo 2007), which might obscure any effects of inbreeding on competitive effort and investment in current reproduction. Potentially, the absence of an opposite sex resident on the carcass could alter the incentive for intruders to attempt a takeover (Trumbo 2007). However, a pilot study ($n = 10$) demonstrated that infanticidal takeovers still occurred in the absence of a female. We excluded all cases where no larvae had hatched ($n = 23$). These cases are excluded from the final sample sizes for each treatment given above.

We introduced the intruder by placing him near the carcass, using a clean pair of forceps. In the control treatments, we removed the lid of the container and disturbed the soil with forceps but without introducing an intruder. Males were

then left for 72 h, after which time we recorded whether a takeover had occurred. A pilot study ($n = 10$) and previous work on *Nicrophorus orbicollis* (a closely related species; Trumbo 2007) suggested that 72 h allowed residents and intruders sufficient time to interact and settle the dispute over ownership of the carcass. The occurrence of a successful defense or takeover was based on information on the proximity of the resident and intruder to the carcass and the presence or absence of larvae on the carcass (Trumbo 2007). Whenever the resident was in close proximity to the carcass, larvae were still present on the carcass, and the intruder was away from the carcass, this was scored as successful defense of the carcass. However, if the intruder was in close proximity to the carcass, the resident was away from the carcass, and no larvae were present on the carcass, this was scored as a successful takeover. In one trial, the intruder was on top of the carcass while the resident was buried in the soil in a far corner of the container and some larvae were still present on the carcass. This trial was scored as a successful takeover because the intruder was observed killing and eating the larvae. In all other trials in which the intruder was in close proximity to the carcass ($n = 21$), there were no surviving larvae on the carcass.

At the end of the 72 h, we also checked all males for any injuries (missing legs and/or antennae) and weighed them to obtain their posttrial mass, from which a change in mass across the experimental trial could be calculated. The males were then transferred to individual transparent plastic containers (11 cm \times 11 cm \times 3 cm) filled with moist soil and fed organic beef twice a week. To record life span, we checked males three times a week and recorded the date of death. In those trials where larvae were still present when the males were removed ($n = 93$), the larvae were left until they dispersed from the carcass (usually 1–2 days after the males were removed), at which point we counted the larvae and weighed the brood. We calculated the average larval mass in a brood by dividing the brood mass by the number of larvae. Larvae were approximately 1 day old when the intruder was introduced, and they spent an additional 4–5 days with the resident before dispersal from the carcass. This is in keeping with the amount of time parents spend with larvae in this species (Eggert et al. 1998). Removing the caring parent 1–2 days before dispersal has no effect on larval growth or survival under laboratory conditions (Eggert et al. 1998).

Statistical Analysis

All data were analyzed in R (ver. 3.1.3). Data on the outcome of trials (takeover, no takeover) and injuries to residents and intruders (injured, uninjured) were analyzed using binary logistic regression, with the relative difference in body size between residents and intruders included in all

Table 1: Model for occurrence of infanticidal takeovers of a carcass defended by an inbred or outbred male resident against an inbred or outbred male intruder

Factor	Estimate	SE	<i>z</i>	<i>P</i>
Own status	.87	1.28	.68	.49
Opponent's status	3.07	1.18	2.59	.0095
Own × opponent's status	−.87	1.44	−.60	.55
Relative size difference	.55	1.43	.38	.70

Note: Factors include the resident's own status (inbred, outbred), the opponent's status (inbred, outbred), the interaction between the two, and the relative size difference between the resident and intruder. We provide information on the parameter estimates, standard errors, test statistics (*z* values), and *P* values. The reference category was outbred for the resident's own status and opponent's status. Data were analyzed using binary logistic regression. Significant *P* values are in bold.

models. Data on breeding success (i.e., brood size, mean larval mass) and future reproductive effort (i.e., change in body mass, posteclosion life span) were analyzed using general linear models for traits that had a normal error structure (brood size, average larval mass, change in body mass) and generalized linear models for traits that had a negative binomial error distribution (posteclosion life span). All data are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.p31vn> (Richardson and Smiseth 2017).

Results

Takeovers

Given that males never abandoned their carcasses or cannibalized their own offspring in the two control treatments, we excluded these treatments from analyses of takeovers. Inbred intruders were more likely to take over a carcass from a size-matched resident than outbred intruders (table 1; fig. 1A). However, inbred and outbred residents were equally successful at retaining the possession of the carcass when challenged by a size-matched intruder, and the interaction between the inbreeding status (inbred or outbred) of the resident and the intruder had no effect on the probability of takeovers (table 1). Furthermore, the probability of a takeover was independent of the relative size difference between the resident and the intruder, as expected given that the two males were matched for size (table 1). In sum, the only factor influencing the probability of takeovers was the intruder's inbreeding status.

Injuries

Injuries never occurred in the two control treatments, and we therefore excluded these treatments from our analyses on the occurrence of injuries. Inbred males were injured more often than outbred males as both residents and intruders

(table 2; fig. 1B, 1C). However, there was no effect of the interaction between the inbreeding status of the resident and the intruder on the likelihood of injuries (table 2). Thus, the only factor that influenced the risk of injury to a male was its own inbreeding status, and there was no evidence that inbred males were injured more often when competing against an outbred male.

Potentially, variation in the injury status of the two males might influence the likelihood of a successful takeover by the intruder. However, when including injury status in the model, we found no evidence that the injury status of either

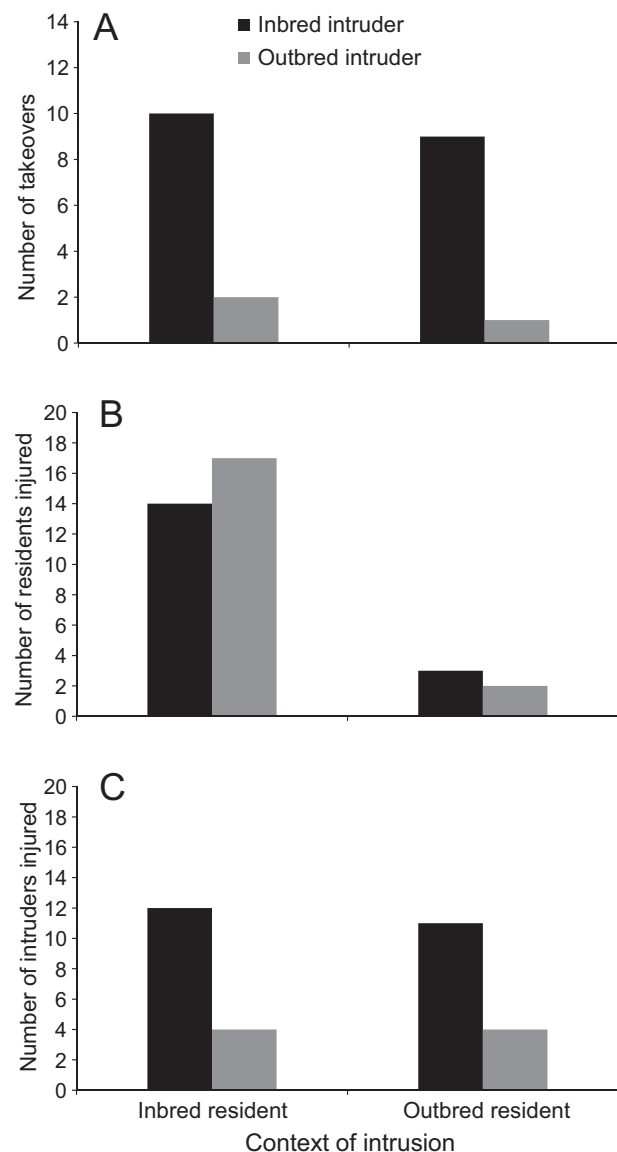


Figure 1: Number of trials (out of 77) that ended in infanticidal takeover (A), injury to the resident (B), or injury to the intruder (C) when an inbred or outbred resident was challenged by an inbred (black bars) or outbred (gray bars) intruder.

Table 2: Model for risk of injury to either the male resident or the male intruder

Factor	Estimate	SE	<i>z</i>	<i>P</i>
Resident:				
Own status	4.35	1.07	4.07	<.0001
Opponent's status	.62	1.03	.60	.55
Own × opponent's status	−1.90	1.33	−1.42	.15
Relative size difference	.16	1.52	.11	.92
Intruder:				
Own status	2.08	.81	2.56	.010
Opponent's status	.18	.81	.22	.82
Own × opponent's status	−.23	1.05	−.22	.83
Relative size difference	1.00	1.23	.82	.41

Note: Factors include the male's own status (inbred, outbred), the opponent's status (inbred, outbred), the interaction between the two, and the relative size difference between the resident and the intruder. We provide information on the parameter estimates, standard errors, test statistics (*z* values), and *P* values. The reference category was outbred for inbreeding status and uninjured for injury status. Data were analyzed using binary logistic regression. Significant *P* values are in bold.

the resident (estimate = 0.18, SE = 0.50, *z* = 0.359, *P* = .719) or the intruder (estimate = 0.033, SE = 0.51, *z* = 0.064, *P* = .949) influenced the likelihood of takeovers by the intruder.

Breeding Success

We analyzed data on breeding success (brood size and mean larval mass at dispersal) for males in the two control treatments (inbred and outbred residents breeding in the absence of an intruder) and for those residents in the experimental treatments that retained possession of the carcass when challenged by a size-matched intruder. There was no main effect of the resident's own inbreeding status on either brood size or mean larval mass (table 3; fig. 2). In contrast, the status of the intruder (inbred, outbred, or absent) had a highly significant effect on the breeding success of residents (table 3; fig. 2). Residents produced a smaller brood size comprised of lighter larvae when challenged by an inbred intruder as opposed to when challenged by an outbred intruder or not challenged by an intruder (table 3; fig. 2). These results suggest that residents suffered a substantial fitness cost when defending their brood against an inbred intruder. There was no evidence that the resident's own body size had an effect on either brood size or mean larval mass (table 3).

Change in Mass and Life Span

Inbred residents gained less mass while breeding than outbred residents (table 4; fig. 3A), suggesting that inbred

males acquired fewer resources from the carcass for investment in future reproduction. Residents also gained less mass when the opponent was inbred as opposed to when the intruder was outbred or there was no intruder (table 4; fig. 3A). For intruders, there was no evidence that either their own inbreeding status or the inbreeding status of the opponent influenced their mass gain (table 4; fig. 3B). Furthermore, there was no evidence that their own initial body size influenced the mass gain of either residents or intruders (table 4). Residents gained significantly more mass over the experimental trial than did intruders (estimate = −10.51, SE = 4.29, *t* = −2.45, *P* = .0015; mean ± SE change in mass for residents [g]: 0.034 ± 0.0024; mean ± SE change in mass for intruders [g]: 0.021 ± 0.0036), presumably reflecting that residents had more time to feed on the carcass.

Inbred males had a significantly shorter posteclosion life span than outbred males both as residents and as intruders (table 4; fig. 3C, 3D). There was no evidence that the opponent's status influenced the life span of either residents or intruders (table 4; fig. 3C, 3D). Furthermore, there was no evidence that the male's body size influenced its life span (table 4). Given that injury status (injured, uninjured) might influence life span, we repeated the analyses after adding information on injury status to the model. Injured beetles had significantly shorter life spans than uninjured beetles, potentially as a consequence of damage or infection (estimate = −0.084, SE = 0.042, *z* = −1.98, *P* = .046; mean ± SE life span for uninjured males [days]: 59 ± 1; mean ± SE life span for injured males [days]: 48 ± 1). However, there was no effect of the interaction between inbreeding status and injury status on life span (estimate = 0.14, SE = 0.12, *z* = 1.16, *P* = .24). There was no significant difference in the posteclosion life span of residents and intruders (estimate = 0.0077, SE = 0.035, *z* = 0.22, *P* = .83; mean ± SE life span for residents [days]: 56 ± 1; mean ± SE life span for intruders [days]: 55 ± 2).

Discussion

We found that inbred intruders were more successful at taking over a carcass from a resident male than outbred intruders and that inbred males were injured more frequently than outbred males both as residents and as intruders. These results confirm our prediction that inbred males increased their competitive effort and suggest that this was associated with a higher risk of injury to inbred males. Our results contradict previous work reporting that inbred individuals are weaker competitors than outbred ones (Sharp 1984; Meagher et al. 2000; Haag et al. 2002; Höglund et al. 2002; Hoffman et al. 2004; Välimäki et al. 2007). Our results also show that the risk of injury to a male was influenced only by the male's own inbreeding status

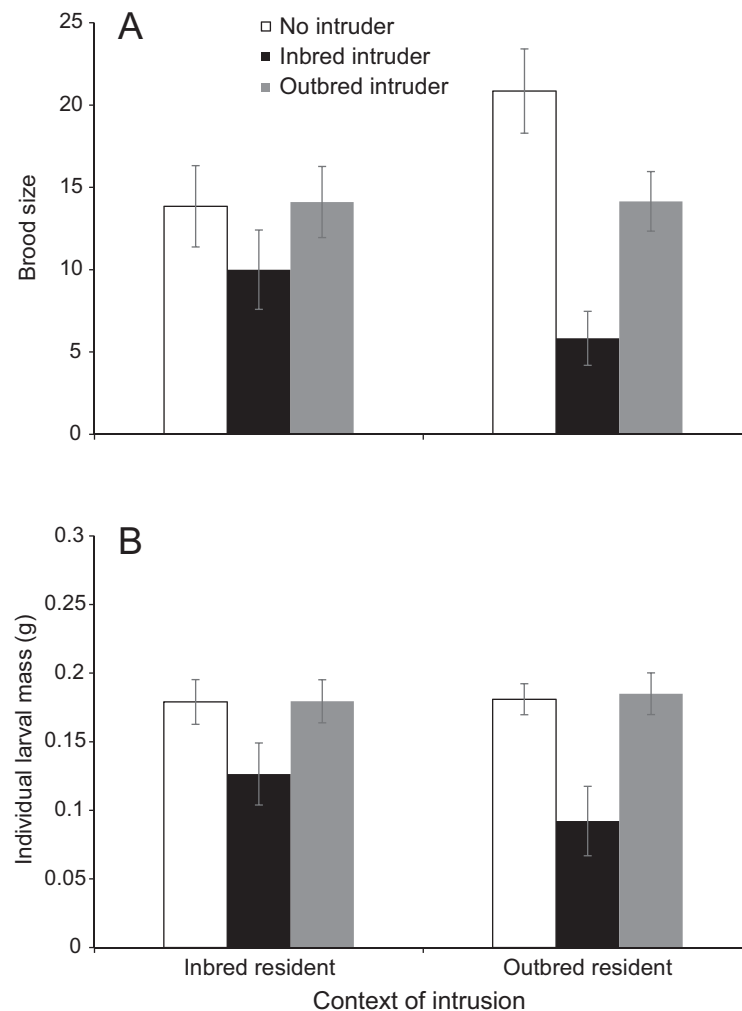


Figure 2: Means \pm SEs for brood size at dispersal (A) and individual mass of larvae at dispersal (B) for inbred and outbred residents when challenged by no intruder (white bars), an inbred intruder (black bars), or an outbred intruder (gray bars).

and that inbred males were as likely to be injured by an inbred opponent as by an outbred opponent. Thus, there was no evidence that inbred males were injured more often when competing against an outbred opponent, as would be expected if inbred males were weaker competitors. We therefore conclude that inbred males took greater risks than outbred males when either defending their brood or attempting to take over a carcass. Our study may provide evidence for increased competitive effort by inbred males because we used an experimental design in which we matched inbred and outbred males for size, thereby excluding any effects due to asymmetries in body size. There is no evidence for a difference in average body size of inbred and outbred individuals in *Nicrophorus vespilloides* (Mattey et al. 2013; Pilakouta et al. 2015), and our design is therefore appropriate for this species.

In contrast to what we predicted, we found no evidence that inbred male residents were more successful at defending the brood against intruders than outbred residents. Thus, our results suggest that inbreeding had a differential effect on intruders and residents, increasing the competitive effort of the former but having no impact on the latter. One possible explanation for this is that the value of the brood to a resident increases over time as the offspring age (Trumbo and Valletta 2007), irrespective of whether the resident is inbred or outbred. In contrast, the value of a used carcass to an intruder may diminish over time as the larvae consume more of the carcass (Trumbo 2007); inbred and outbred males may value this decline differently if inbred males have a lower future reproductive potential than outbred ones. Alternatively, inbred males might have less energy for allocation to competition than outbred males, given that inbred

Table 3: Model for breeding success (brood size and mean larval mass at dispersal) for residents

Factor	Brood size				Mean larval mass			
	Estimate	SE	<i>t</i>	<i>P</i>	Estimate	SE	<i>t</i>	<i>P</i>
Own status	.52	2.95	.175	.86	-.0048	.028	-.17	.86
Opponent's status	-7.64	3.02	-2.53	.014	-.092	.029	-3.09	.0027
Contrast:								
Inbred vs. outbred	8.31	3.19	2.61	.010	.092	.026	3.56	.0005
Inbred vs. absent	15.0	3.19	4.71	<.0001	.088	.026	3.41	.008
Own × opponent's status	3.85	4.12	.93	.35	.039	.040	.97	.33
Own body size	2.13	2.44	.89	.38	.027	.024	.11	.91

Note: Factors include the resident's own status (inbred, outbred), the opponent's status (inbred, outbred, absent), the interaction between the two, and the resident's own body size. We provide information on the parameter estimates, standard errors, test statistics (*t* values), and *P* values. Data were analyzed using general linear models. The reference category was outbred for own and opponent's inbreeding status and inbred when performing post hoc contrasts. Significant *P* values are in bold.

residents gained less mass than outbred residents. If so, any increase in competitive effort by inbred residents might be counterbalanced by a lower mass gain by inbred residents.

There are at least three alternative explanations for why inbred males might increase their competitive effort in re-

sponse to higher mortality rates. First, inbreeding might induce terminal investment because of the lower future reproductive potential of inbred males. Such a response might have evolved specifically in response to inbreeding or could reflect a general response to an overall decline in condition

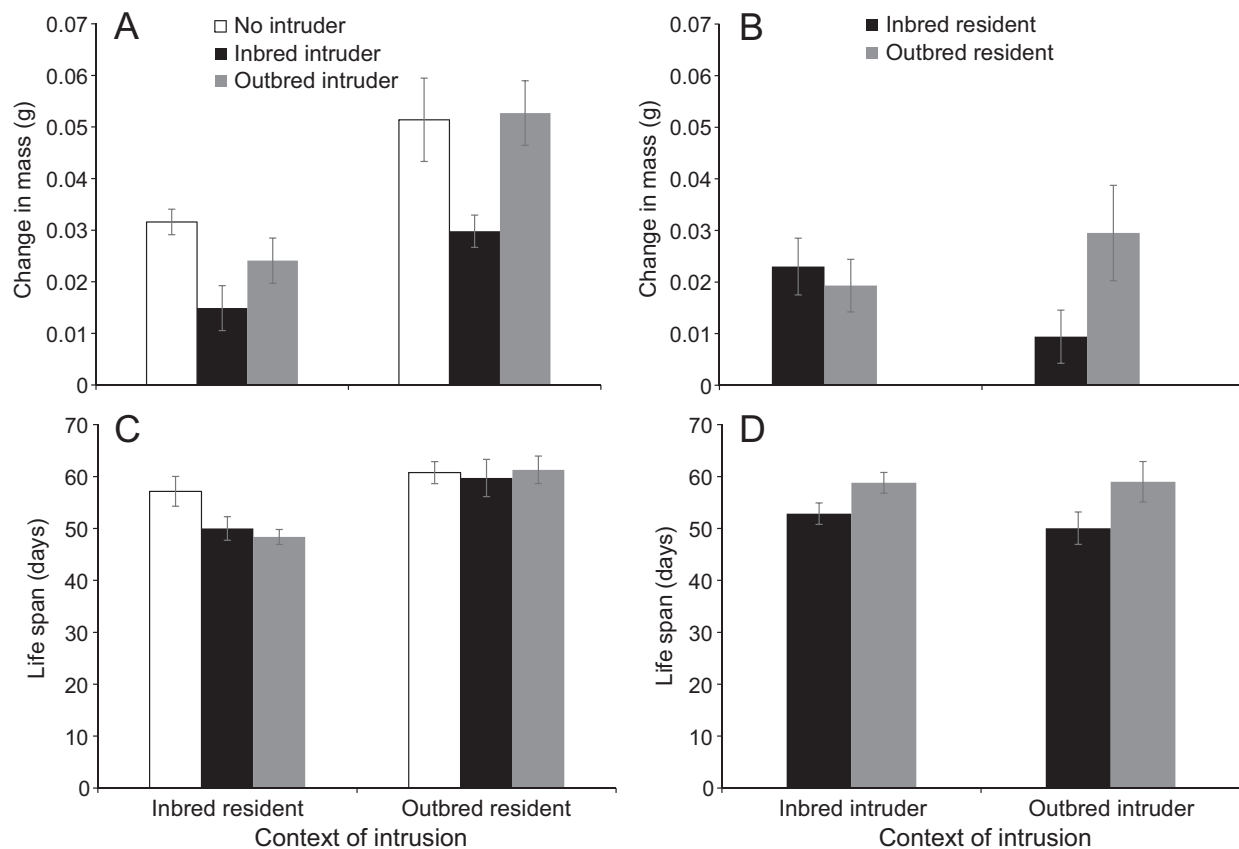


Figure 3: A, Change in mass for residents when facing no intruder (white bars), an inbred intruder (black bars), or an outbred intruder (gray bars). B, Change in mass for intruders facing either an inbred resident (black bars) or an outbred resident (gray bars). C, Posteclosion life span for residents facing no intruder (empty bars), an inbred intruder (black bars), or an outbred intruder (gray bars). D, Posteclosion life span for intruders facing either an inbred resident (black bars) or an outbred resident (gray bars). Values are means \pm SEs.

Table 4: Model for components of future reproductive effort (change in mass and posteclosion life span) for male residents and male intruders

Factor	Change in mass				Posteclosion life span			
	Estimate	SE	<i>t</i>	<i>P</i>	Estimate	SE	<i>z</i>	<i>P</i>
Residents:								
Own status	−26.14	7.24	−3.61	.00046	−.255	.063	−4.04	<.0001
Opponent's status	−19.96	7.38	−2.70	.0079	−.047	.062	−.76	.44
Contrast:								
Inbred vs. outbred	20.66	6.96	2.96	.0041	.040	.066	.61	.54
Inbred vs. absent	21.17	7.00	3.02	.0035	.018	.066	.28	.77
Own × opponent's status	12.07	10.18	1.18	.23	.071	.088	.79	.42
Own body size	9.39	4.86	1.93	.060	−.069	.042	−1.66	.10
Intruders:								
Own status	−9.21	10.19	−.90	.36	−.16	.076	−2.14	.032
Opponent's status	−17.95	9.78	−1.83	.071	−.0039	.074	−.053	.95
Own × opponent's status	23.76	14.53	1.64	.11	.058	.11	.54	.58
Own body size	12.18	9.23	1.32	.19	−.0022	.069	−.032	.97

Note: Factors include the resident's or intruder's own status (inbred, outbred), status of the opponent (inbred, outbred, absent), the interaction between the two, and their own body size. We provide information on the parameter estimates, standard errors, test statistics (*t/z* values), and *P* values. The reference category was outbred for own and opponent's status and inbred when performing post hoc contrasts. Data for mass gain were analyzed using a general linear model. Data for life span were analyzed using a generalized linear model fitted with a negative binomial error distribution. Significant *P* values are in bold.

or health associated with inbreeding. Previous studies on this species provide evidence for terminal investment in response to a decline in health status (Cotter et al. 2011), suggesting that inbreeding could trigger terminal investment as a result of declining health status. Second, inbred males might increase their competitive effort if inbreeding depression alters the trade-off between current and future reproduction. If inbred individuals reduce their investment in future reproduction (e.g., life span), we might expect a corresponding increase in investment in current reproduction (assuming all else being equal) because of the trade-off between future and current reproduction (Stearns 1992). Third, inbred males might have a higher competitive effort because of selective disappearance of inbred males during juvenile development. Inbred individuals have higher mortality during juvenile development (Mattey et al. 2013), but it is unclear whether such selective disappearance is biased against males with a low competitive effort as adults.

We found that male residents produced smaller broods comprised of lighter offspring when confronted by an inbred intruder. This suggests that inbred intruders impose a greater cost to resident males than outbred intruders. Pre-

sumably, inbred males are more persistent or aggressive when attacking the resident, thereby forcing the resident male to spend more time or energy repelling inbred intruders, with detrimental effects on its offspring's survival and growth. Thus, our results provide evidence for indirect genetic effects (Moore et al. 1997) in the context of inbreeding (Mattey et al. 2013; Mattey and Smiseth 2015a). The presence of such indirect genetic effects has important implications because we would need to consider potential fitness consequences to outbred individuals interacting with inbred ones when estimating the true fitness costs of inbreeding. Previous work shows that outbred individuals suffer fitness costs because of interactions with inbred individuals when outbred offspring receive care from inbred parents (Mattey et al. 2013) and when outbred females mate with inbred males (Okada et al. 2011). Thus, our results suggest that such costs might occur in a wide range of contexts where outbred individuals interact with inbred ones. Furthermore, our results suggest that we need to consider how to estimate the coefficient of inbreeding depression. Traditionally, this coefficient is calculated by comparing the reduction in fitness of inbred individuals relative to the fitness of outbred individuals in

Table 5: Coefficients of inbreeding depression (δ) for brood size and mean larval mass when inbred individuals are compared either with outbred individuals interacting with inbred individuals or with outbred individuals interacting with other outbred individuals

Traits	δ when outbred individuals interact with inbred ones	δ when outbred individuals interact with outbred ones
Brood size	−1.16	.11
Mean larval mass	−.74	.15

a population (Hedrick and Kalinowski 2000). If outbred individuals suffer a fitness cost because of interactions with inbred individuals, we might underestimate the coefficient of inbreeding depression if we compare inbred individuals with outbred individuals interacting with inbred individuals rather than with other outbred individuals. To illustrate this point, we estimated coefficients of inbreeding depression on the basis of comparisons either with outbred males interacting with inbred ones or with outbred males interacting with other outbred males. In this case, there was evidence for inbreeding depression only when inbred males were compared with outbred males interacting with other outbred males (table 5). These results highlight the importance of considering which cohort of outbred individuals to use as a comparison with inbred ones when calculating coefficients of inbreeding depression.

We found that inbred residents gained less mass than outbred residents during their breeding attempt. Furthermore, male residents gained less mass when the opponent was inbred as opposed to when the intruder was outbred or there was no intruder. Mass gain during breeding is due to personal consumption from the carcass and provides an indicator of increased allocation of resources to somatic maintenance and thus future reproduction (Creighton et al. 2009; Billman et al. 2014). Our findings suggest that inbred residents invest less in future reproduction than outbred residents and that male residents suffer a cost in terms of personal consumption from the carcass when facing an inbred intruder. Finally, we found that inbred males had a shorter life span than outbred males both as residents and as intruders. These findings are consistent with a recent study on the same species showing that inbred males provide more indirect care than outbred males (Mattey and Smiseth 2015a).

In summary, we have shown that inbred males increased their competitive effort and that opponents of inbred male intruders suffered a substantial fitness cost because of the increased competitive effort by the latter. Our results have important wider implications for our understanding of the interaction between intraspecific competition and inbreeding depression. First, in order to estimate the true fitness costs of inbreeding to a population, we need to consider potential detrimental effects to outbred individuals interacting with inbred ones. Second, when estimating the coefficient of inbreeding depression, we need to carefully consider whether to compare the fitness of inbred males with (1) outbred males interacting with inbred males or (2) outbred males interacting with other outbred males.

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Nicrophorus vespilloides feeding larvae. Photo credit: Per T. Smiseth.